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1
Ser Ser Ser Phe Asp Lys Gly Lys Tyr Lys Lys Gly Asp Asp

15
Ala Ser Tyr Phe Glu Pro Thr Gly Pro Tyr Leu Met Val Asn

29
Val Thr Gly Val Asp Ser Lys Gly Asn Glu Leu Leu Ser Pro

42
His Tyr Val Glu Phe Pro Ile Lys Pro Gly Thr Thr Leu Thr

57
Lys Glu Lys Ile Glu Tyr Tyr Val Glu Trp Ala Leu Asp Ala

□ : single amino acid to Ala substitution
○ : combined (2 or 3) amino acid to Ala substitutions

71
Thr Ala Trp Lys Glu Phe Asn Val Val Glu Leu Asp Pro Ser

85
Ala Lys Ile Glu Val Thr Tyr Tyr Asn Lys Asn Lys Lys Lys

99
Glu Glu Thr Lys Ser Phe Pro Ile Thr Glu Lys Gly Phe Val

113
Val Pro Asn Leu Ser Glu His Ile Lys Asn Pro Gly Phe Asn

127
Leu Ile Thr Lys Val Val Ile Glu Lys Lys

136

□ : single amino acid to Ala substitution
○ : combined (2 or 3) amino acid to Ala substitutions

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/31 C07K14/31 A61K38/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	COLLEN, D. ET AL: "Thrombolytic properties of poorly immunogenic variants of recombinant staphylokinase." FIBRINOLYSIS & PROTEOLYSIS, (JUNE, 1998) VOL. 12, NO. SUPPL. 1, PP. 30. MEETING INFO.: XIVTH INTERNATIONAL CONGRESS ON FIBRINOLYSIS AND THROMBOLYSIS LJUBLJANA, SLOVENIA JUNE 22-26, 1998, XP002111034 abstract	7,8
X	COLLEN D ET AL: "Recombinant staphylokinase variants with altered immunoreactivity. III: Species variability of antibody binding patterns." CIRCULATION, (1997 JAN 21) 95 (2) 455-62. , XP002111035 page 456; tables 2,3 -/-	7,23-27

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"Z" document member of the same patent family

Date of the actual completion of the international search

2 August 1999

Date of mailing of the international search report

11.08.99

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COLLEN D ET AL: "Recombinant staphylokinase variants with altered immunoreactivity. II: Thrombolytic properties and antibody induction." CIRCULATION, (1996 JUL 15) 94 (2) 207-16. , XP002111036 page 214 - page 215 ---	7,23-27
X	COLLEN D ET AL: "Recombinant staphylokinase variants with altered immunoreactivity. I: Construction and characterization." CIRCULATION, (1996 JUL 15) 94 (2) 197-206. , XP002111037 table 3 ---	7,23-27
X	COLLEN D ET AL: "Recombinant staphylokinase variants with altered immunoreactivity. IV: Identification of variants with reduced antibody induction but intact potency." CIRCULATION, (1997 JAN 21) 95 (2) 463-72. , XP002111038 page 463 ---	7,23-27
X	EP 0 721 982 A (LEUVEN RES & DEV VZW ;COLLEN DESIRE JOSE (BE)) 17 July 1996 (1996-07-17) example 2 -----	7,23-27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 99/00748

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 1-6, and in part 7, 10-14, 23-27
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-6, and in part 7,10-14,23-27

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The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1.1). Present claim 1 relate to staphylokinase derivatives defined by reference to a desirable characteristic or property, namely to staphylokinase derivatives showing a reduced immunogenicity as compared to wild-type staphylokinase, after administration to patients with arterial thrombosis.

The claims cover all staphylokinase derivatives having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such products. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

1.2). Present claims 2-6,10-14 relate to an extremely large number of possible staphylokinase derivatives, and claims 24 and 25 relate to an extremely large number of methods.

For instance, claims 2-4 relate to staphylokinase derivatives having essentially the amino acid sequence as depicted in figure 1 in which one or more amino acids have been replaced by another amino acid thus reducing the reactivity with a panel of murine monoclonal antibodies (claim 2), or thus reducing the absorption of SakSTAR-specific antibodies from plasma of patients treated with staphylokinase (claim 3), or without reducing the specific activity by more than 50 percent (claim 4).

Claim 6 relates to staphylokinase derivatives listed in Tables 1-8,13,19, and 20 having the amino acid sequence as depicted in figure 1 in which the indicated amino acids have been replaced by other amino acids thus reducing the absorption of SakSTAR-specific antibodies.. without reducing the specific activity.

The staphylokinase derivatives of claim 10 are the derivatives of claims 1-9 and, further, having an amino acid substituted with Cys, resulting in dimerization and/or increase specific activity and/or reduced clearance and/or increased thrombolytic potency.

The staphylokinase derivatives of claim 11 are the derivatives of claims 1-10 with polyethylene glycol (PEG) substitution, characterized by a maintained specific activity and a significantly reduced plasma clearance. A similar functional limitation is given for claim 13.

In fact, the claims contain so many options and for the method claims so many possible mutated DNA fragments to be expressed that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible.

Moreover, the attention of the applicant is drawn to the fact that the further functional characterization (i.e. aim to be achieved) given within said claims 4-6,10,11, and 13 is not suitable to render the scope of said claims clear (Art. 6 PCT).

1.3). Present claim 7 relates to an extremely large number of possible staphylokinase derivatives. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the products claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

be supported and disclosed, namely those parts relating to the following staphylokinase derivatives or combination variants of SakSTAR and apparently having the desired properties, namely reduced immunogenicity and thrombolytic efficacy:

- SakSTAR (K74A,E75A,R77A),
- SakSTAR (E80A,D82A),
- SakSTAR (E75A),
- SakSTAR (K35A,E75A),
- SakSTAR (E80A),
- SakSTAR (D82A),
- SakSTAR (E75A,D82A),
- SakSTAR (K35A),
- SakSTAR (G36A),
- SakSTAR (K130A),
- SakSTAR (V132A),
- SakSTAR (K74Q),
- SakSTAR (K130T),
- SakSTAR (V132R),
- SakSTAR (K130T,K135R),
- SakSTAR (E65Q,K74Q,K130T,K135R),
- SakSTAR (E65A,K74Q,K130T,K135R),
- SakSTAR (E80A,D82A,K130T,K135R),
- SakSTAR (K74R,E80A,D82A,K130T,K135R),
- SakSTAR (K74Q,E80A,D82A,K130T,K135R),
- SakSTAR (E65D,K74Q,E80A,D82A,K130T,K135R),
- SakSTAR (K35A,E65D,K74Q,E80A,D82A,K130T,K135R),
- SakSTAR (E65Q,K74Q,N95A,E118A,K130A,K135R,K136A,+137K),
- SakSTAR (E65D,K74R,E80A,D82A,K130T,K135R),
- SakSTAR (E65S,K74R,E80A,D82A,K130T,K135R),

1.4). The search has been carried out for staphylokinase derivatives having an amino acid substituted with Cys or with PEG substitution (claims 10-14), in so far as these derivatives relate back to the above specifically mentioned staphylokinase derivatives.

The above comment also applies for claims 23-27.

2). The search has been carried out for all of the above mentioned derivatives and variants although the present international application lacks in principle unity of invention, since certain of the above mentioned SakSTAR derivatives were already known from the prior art. Therefore, there exists no longer a technical relationship between the different staphylokinase derivatives of claim 7.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 99/00748

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0721982 A	17-07-1996	AU 705119 B	13-05-1999
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		EP 0721013 A	10-07-1996
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